two alternative arrangements with two stems per dimer (Chen and Wallis, 2001; Teillet et al., 2008). Clearly, further biochemical studies are required to understand the activation mechanism, but at least we are no longer in the dark about a critical connection in the MBL-MASP assembly.

ACKNOWLEDGMENTS

E.H. is a Wellcome Trust Senior Research Fellow.

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Type II ABC Permeases: Are They Really So Different?

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DOI 10.1016/j.str.2011.10.007

Structural and biochemical data reported by Tirado-Lee et al. (2011) in this issue of *Structure* reveal the existence of high and low affinity ABC transporters for the same substrate in a single organism, thus raising questions about structural and mechanistic differences within the ABC superfamily.

ATP binding-cassette (ABC) transporters couple ATP hydrolysis to vectorial movement of substrates across cell membranes. Of ancient origin and ubiquitous occurrence, they have been adapted to traffic a vast array of compounds, performing roles such as nutrient import and removal of toxins, antigen presentation, hormone release, signal reception, channel gating, and many others (Higgins, 1992; Jones and George, 2004). Their central roles in many physiological process has brought ABC transporters to the forefront in biomedical research in diverse areas including multidrug resistance in cancers and human genetic disorders, such as cystic fibrosis.

The conserved core architecture comprises two transmembrane domains (TMDs) that form the translocation conduit and contain the substrate binding site(s), and two nucleotide binding domains (NBDs) that form two ATP-binding sites that hydrolyse ATP cooperatively. Bacterial importers have an associated periplasmic substrate binding protein (PBP) that delivers the substrate to the TMDs. The general architecture of all PBPs consists of two globular domains, with a binding pocket located in the cleft formed between them. While the NBDs are highly conserved in sequence and structure, the sequences of the TMDs are not, reflecting their role in binding and forming a channel for diverse substrates.

Crystallographic analyses in recent years have produced three radically different architectures for the TMDs of ABC transporters (Jones et al., 2009); one for multidrug resistance-type exporters, and two for bacterial importers, the latter designated types I and II. This result was unexpected, since phylogenetic analyses of the PBPs and NBDs are closely correlated, giving no indication of divergence in their intervening cognate TMDs (Saurin et al., 1999). In addition, despite the existence of numerous NBD crystal structures, no systematic differences between the NBDs of the different classes of importers have been identified. It thus had been expected that ABC transporters evolved from a single progenitor (Saurin et al., 1999), and the reason for the different structures of the TMDs has been a question of interest (Jones et al., 2009). A seemingly plausible explanation is that different kinds of substrate are more effectively handled by different TMD structures. Indeed, type I structures include the maltose (MalFGK2), molybdate (Mb; ModABC), and methionine (MetNI) systems and type II includes BtuCDF (vitamin B12) and HI1470/71, and the structural classification correlates with the phylogenetic classification for these systems, with the two sets of transporters grouped on two distinct branches. In addition, because the previously unknown substrate of HI1470/71 was presumed to be a metal chelate, the structural differences between the type I and II importers also

appeared to correlate broadly with differences in their substrates.

Enter Tirado-Lee et al. (2011) in the current issue of *Structure*. They describe high-resolution structures for the tungstate (Tg)- and Mb-bound PBP of an ABC importer. In this report, the authors identify the substrate of the type II importer $MolAB_2C_2$ (formerly HI1470/71/72), for which the TMD structure is known, as being the same as for the type I ModABC Mb/Tg importer. This shows that the substrate per se is not the source of the differences in ABC transporter architectures. These authors (Tirado-Lee et al., 2011) show that the MolA PBP belongs to the class III PBPs and has an affinity for Mb and Tg significantly lower than the ModA PBP. This indicates, for the first time, the existence of high and low affinity systems for Mb/Tg in a single organism. Moreover, the authors find that other oxyanions or iron chelates are not substrates for MolA.

Why have two ABC transport systems with different affinities for the same substrate in the same organism? One possibility might be to take advantage of situations where either the ambient concentration of substrate is high or the requirement for it is low by expending less energy in its uptake. For any ABC importer, there will be an optimal concentration of transporters in the membrane and PBPs in the periplasmic space to allow the two to engage at a rate that enables effective transport. As the concentration of the substrate increases or the requirement for it diminishes, there will come a point at which it is more efficient to employ lower copy numbers of a transporter in which the PBPs remains attached. Indeed, for two type II importers, the vitamin B_{12} importers BtuCD-F and MolABC, in contrast to type I importers, the PBPs form an extremely stable complex with the TMD. For example, although for BtuF nucleotide did increase dissociation, the overall K_d in the presence of nucleotide was six orders of magnitude less than for the type I methionine importer (Lewinson et al., 2010).

Structural analysis indicated significant mechanistic differences between type I and type II importers. Thus, type II importers are suggested to couple the opening and closing of the NBD dimer to inward- and outward-facing conformations of the TMDs in a manner converse to that in type I importers, and, also in contrast to type I, to employ a mechanism that involves asymmetry in the TMDs and lateral or "sheering" motions between the NBDs. The new study by Tirado-Lee et al. (2011) and other recent studies characterizing type II import systems have interpreted their results in support of significant mechanistic differences between these and type I systems. Thus, the class III PBPs, which include BtuF and MolA, are expected, on the basis of structural analysis, to be relatively rigid and not open significantly to bind and release substrate, as do the class II PBPs, such as ModA. Nevertheless, contrary to this idea, MD simulations and elastic network analysis indicated that the "Venus flytrap" domain opening and closing mechanism was likely to be common to all PBPs, including those with the class III structure (Kandt et al., 2006). The observation that high concentrations of substrate caused dissociation of BtuF from the complex was also suggested to indicate mechanistic differences with the type I importers (Lewinson et al., 2010). However, at physiological concentrations of substrate, BtuF binds strongly to the transporter, but, intriguingly, as the ratio of substrate to PBP reaches and passes a stoichiometry of 1:2, there is a step-like decrease in PBP transporter binding.

In the study by Tirado-Lee et al. (2011), the stoichiometry of binding of Mb and Tg to MolA is 0.67 and 0.57 respectively, close to a 1:2 ratio. What may we infer from these data? One possibility is that the PBPs form dimers that bind only one substrate molecule at any one time. PBPs generally are expressed at a stoichiometry of 2 per transporter and, in some cases, are covalently attached. Cooperativity between the PBPs in transport has been observed for attached (OpuA) (Biemans-Oldehinkel and Poolman, 2003) and free PBPs (maltose permease) (Manson et al., 1985). Recently, extensive characterization of the kinetics of association of the PBP with the transporter for BtuCD, MolABC, and MetNI found all showed clear biphasic characteristics (Lewinson et al., 2010), consistent with the notion that the transporter interacts with two PBPs. Notably, substrate-bound (Borths et al., 2002; Karpowich et al., 2001) and unliganded (Karpowich et al., 2001) BtuF were observed to form identical dimers in three separate crystals, all with distinct lattice arrangements, suggesting the dimer is not an artifact of crystal packing. Moreover, two other class III PBPs, TroA (Lee et al., 1999) and AdcAII (Loisel et al., 2008), were also observed to form a non-crystallographic dimer in the substrate-bound form. Significantly, in the TroA, AdcAII, and BtuF dimers, the dimer interfaces are equivalent in being formed predominantly by a variable loop that joins a structurally conserved β strand and α-helix in the N-terminal lobe. In the new structure of MolA and all other class III PBP structures, the equivalent region is of variable length, sequence, and structure and generally contains sections with high temperature factors, compatible with the possibility that it forms a docking and interaction interface. While the overall folds of MolA, BtuF, and TroA are similar, the authors show how the binding pocket is modified to accommodate significantly different-sized substrates.

The prevailing paradigm for the function of ABC transporters is the Jardetsky-adapted two-state model in which a single transmembrane channel alternates between inward- and outward-facing conformations, coupled to opening and closing of the NBD dimer. Some significant data, nevertheless, are at odds with this idea, and a constant contact, alternating hydrolysis mechanism for the NBD function, has also been suggested. This latter model appears incompatible with the two-state idea, and thus the whole transporter structures. In addition, as discussed above, although biochemical data has been interpreted as supporting the structural and implicit mechanistic differences between the type I and type II importers, plausible alternative interpretations or contradictory data exist. It thus seems pertinent to pose the question raised by the findings presented by Tirado-Lee et al. (2011) that if substrate specificity is not the basis of the different ABC transporter TMD structures, what, conceivably, might be?

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